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DOI: https://doi.org/10.1017/CBO9781139044547.012

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Ultrasonic vocalizations in house mice: a cryptic mode of acoustic communication

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Introduction

House mice (*Mus musculus*) are thought to communicate primarily through chemosensory signals (see Stopka *et al.*, Chapter 8 in this volume), and yet, like many rodents, house mice also communicate using ultrasonic vocalizations (USVs) (Box 10.1). Zippelius and Schleidt (1956) first studied USVs in house mice when they developed a recording device sufficient to detect ultrasound from pups. Since then, many studies have investigated rodent USVs, concentrating on the occurrence of the calls and the underlying proximate mechanisms. In house mice, USVs are emitted by pups as distress calls (Ehret, 2005) and by adults during courtship (primarily males) (Nyby, 1983) and same-sex social interactions (primarily females) (Costantini and D'Amato, 2006). More than one century ago, song-like features were reported from rare individual house mice, which sang at audible frequencies (Farr, 1857; Coburn, 1912; reviewed in Dice, 1932), and it was predicted that singing might be common in *M. musculus* and would occur at ultrasonic frequencies (Sidebotham, 1877; Dice, 1932). In the 1970s, Gillian Sales, Günter Ehret, and John Nyby and colleagues independently pioneered work on the complexity, proximate control and evolutionary origins of USVs in pups and adult house mice (Sales, 1972b; Ehret, 1975; Nyby *et al.*, 1976). More recently, a detailed, qualitative spectrographic analysis of laboratory mice concluded that male USVs fulfil the criteria for *song* (i.e. a series of syllables, generally more than one type, uttered in succession to form a recognizable sequence or pattern in time (Holy and Guo, 2005)). Amazingly, the playbacks of males' courtship USVs, when slowed down for human hearing, sound like songbirds (http://www.plosbiology.org/article/info:doi/10.1371/journal.pbio.0030386, Audio S1–S5)! We recently found evidence that the courtship USVs of male house mice provide a secondary sexual display (Hoffmann *et al.*, 2009; Musolf *et al.*, 2010) along with chemosensory signals, which appears to be functionally analogous to extravagant ornaments of peacocks and other birds. By

Evolution of the House Mouse, ed. Miloš Macholán, Stuart J. E. Baird, Pavel Munclinger, and Jaroslav Piálek.
Published by Cambridge University Press.

Box 10.1 What is ultrasound? Bioacoustics of USVs

Sound is caused by the detection of a travelling wave; an oscillation of pressure is transmitted through a solid, liquid, or gas and the vibrations are perceived in hearing organs. Ultrasound *per se* is not different from ordinary sound except it is produced at frequencies that exceed the range of human hearing. Humans can perceive frequencies between 20 Hz and 20 kHz, with peak sensitivity at 2 kHz, the so-called 'acoustic frequency' range (Fig. 10.1). Infrasound (frequencies <20 Hz) is used in elephant communication, for example. Ultrasound (frequencies >20 kHz) is used in a variety of animals, such as bats, marine mammals, and rodents (Sales and Pye, 1974).

Most audible sound is produced by vocal fold vibrations under the influence of lung pressure. Due to their pure tone and dependence on a functioning larynx, USVs are thought to be produced by a whistle mechanism in the vocal tract, a hypothesis which has been supported by direct observations of non-vibrating vocal folds during production of these calls (Sanders *et al.*, 2001).

A study of auditory sensitivity in laboratory mouse strains found that the highest hearing sensitivity is around 15 kHz, and a second, subsidiary peak occurs at about 50 kHz (Ehret, 1974). This finding, and further adaptation in sound processing in the brain, indicate that mice have evolved hearing abilities tuned to both their sonic and ultrasonic vocalizations and even specialized their reception of specific ultrasonic calls (Ehret, 2001). Measurements of calling frequency vary among different laboratory strains – it remains to be tested whether these differences are reflected in hearing sensitivity; some laboratory strains do not hear well in the ultrasonic range or are even deaf as adults (Ralls, 1967). Deaf and knock-out mouse strains are useful tools for research on acoustic communication pathways.

Ultrasonic vocalizations, because of their short range, are suitable for communication both inside and outside the burrow. The attenuation inside the burrow depends on wall surfaces – the rougher the structure the greater the attenuation (Fletcher, 1992), whereas outside the burrow the habitat (grass *vs* wood) influences the vocalization range (Smith, 1979). Besides predator evasion through shifting communication in ultrasonic ranges, the ability to perceive ultrasound also might be helpful for mice to detect their enemies. For example, footsteps generate vibrations as well as ultrasonic signatures (Ekimov and Sabatier, 2006, 2008); therefore, mice might use ultrasound for detecting potential predators.

focusing mainly on chemical communication in house mice, researchers have been overlooking an important mode of communication with possible functions in mate choice and social recognition.

The discovery that male house mice emit complex courtship song is stimulating much new research on USVs, and such studies could prove to be as fruitful as the remarkable work on bird song (Marler and Slabbekoorn, 2004). Genetic analyses of USVs are particularly tractable due to the availability of a large number of well-characterized strains of laboratory mice and large amounts of readily available

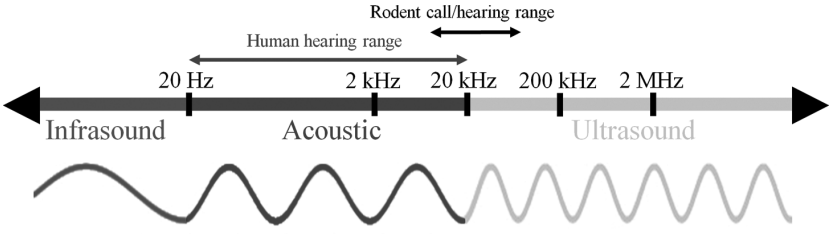

Figure 10. 1 Schematic overview of sound categories defined by frequencies. The human hearing range and the rodent call and hearing range are indicated in the text. See the plate section for a colour version of this figure.

sequence information. USVs of approximately 20 different inbred strains have been studied (reviewed in Hahn and Lavooy, 2005), including MHC-congenic strains (Brown *et al.*, 1999) and hybrids of strain crosses. The differences found among laboratory strains, together with the finding that hybrids (both pups and adults) call more frequently than the pure strains (Maggio and Whitney, 1986; Hahn *et al.*, 1997) indicate that USVs have a genetic basis. The precise role of genetics on USVs can be investigated in greater detail with the availability of an increasing number of mutant, congenic, and transgenic strains (Box 10.2). Combined with behavioural phenotyping, laboratory strains will provide a useful tool to identify and investigate the influence of specific genes on the development and control of USV communication (Crawley, 2000). Such candidate genes could be used to help in understanding the evolutionary origins and functions of USVs in wild house mice and to investigate whether they have a common genetic basis with other rodent species. Almost everything currently known about house mouse USVs comes from studies on inbred laboratory strains, however, and research on USVs in wild or wild-derived mice has only just begun (i.e. Hoffmann *et al.*, 2009, 2012a, b; Musolf *et al.*, 2010).

In this chapter we provide a broad and integrated ethological overview of USVs in house mice and highlight our current knowledge of both the mechanistic (ontogeny and physiology) and evolutionary (functional and phylogenetic) features of this behaviour.

Pup vocalizations

In many rodent species, pups are born blind and deaf, and are incapable of feeding themselves or thermoregulating during their first one or two postnatal weeks. However, at this stage they already produce a variety of sounds, mostly pure ultrasounds (Ehret and Bernecker, 1986; Hahn and Lavooy, 2005) that function to elicit maternal care (Branchi *et al.*, 1998; Hahn and Lavooy, 2005).

Box 10.2 Genetics of USVs in house mice

Our current knowledge on the genetic basis of USVs comes from studies on laboratory mice. Different inbred laboratory strains show distinctive USV patterns. Strains vary in their rates of USV production (low emitters: NZB and C57BL/6J; high emitters: BALB/c, DBA, A/J, BTBR, FVB/NJ), call duration, sound frequency, and bandwidth. The genetic basis of USV emission is corroborated by studies on knock-out and other transgenic mice, which have shown that deletion or insertion of selected genes markedly modifies vocal behaviour (Winslow *et al.*, 2000; Weller *et al.*, 2003; Moles *et al.*, 2004; Shu *et al.*, 2005; Scattoni *et al.*, 2008a, b). A cross-fostering study on laboratory mice pups revealed that call features were primarily dependent on the genotype of the pup, and call amplitude was the only feature dependent on the maternal genotype, i.e. on early environmental factors (Wöhr *et al.*, 2008). Another cross-fostering study with BALB and B6 mice, which show strain-specific call types, revealed that adult courtship calls of fostered males remained similar to calls of their genetic parental strain, and did not copy their fostered parents (Kikusui *et al.*, 2011). This study confirms a genetic influence on USVs and indicates that the strain-specific call types are not socially learned.

Studies investigating ultrasonic vocalization in crosses of mouse strains agree that hybrids always call at higher rates than individuals of the respective parental strain, indicating heterosis and a directional dominance towards specific call parameters (e.g. higher rate of calling, longer calls) (in pups: Hahn *et al.*, 1997, 1998; in adults: Maggio and Whitney, 1986), which may have important fitness values.

Foxp2 is a candidate locus for vocalization and influences sound production in humans, birds, and mice (Teramitsu and White, 2008). It is unknown, however, whether *Foxp2* or a linked receptor influences variation in USVs of adult mice. In contrast to early published reports (Shu *et al.*, 2005), *Foxp2* is not essential for ultrasonic vocalization in mice – recessive homozygotes generate harmonically structured audible calls and complex ultrasounds only under conditions of elevated stress (Fisher and Scharff, 2009). Mutations at *Foxp2* are known to lead to severe reductions in cerebellar growth and postnatal weight gain in mice pups (Groszer *et al.*, 2008), factors that can influence natural behaviours.

A mutant mouse was recently discovered which sang at audible frequencies (A. Uchimura, Osaka University's Graduate School of Frontier Biosciences). Further research with this mutant might also help in understanding the evolution of song and language.

The rate of USV production in house mice increases during the first 6–7 days of life, peaks around day 8, and then starts to decrease until the total disappearance of these calls after 14–16 days (Nyby *et al.*, 1976; Elwood and Keeling, 1982), when pups have grown fur and begin to thermoregulate independently. Pup USVs change during this period both in call length and frequency characteristics (Hahn *et al.*, 1998). Pup and adult calls fall into distinct spectral and temporal categories

(Liu *et al.*, 2003). A recent study followed individual mouse USV development from pup to adult in the CBA/CaJ strain and found that although most (9 of 11) syllable types were already present in the pup repertoire, the proportion and characteristics of each type changed developmentally and the complexity of vocal bouts increased with age (Grimsley *et al.*, 2011). Further studies are needed to identify how these developmental changes occur.

Proximate mechanisms of USV production: ontogeny and physiology

USVs in infant rodents are triggered as part of a general stress reaction, such as cold stress, and are produced through laryngeal braking forced by an abdominal compression reaction aimed at increasing blood flow to the heart (Blumberg and Alberts, 1990). House mouse pups are deaf until the age of 10 days (Ehret, 1983), and variation in call parameters during their development may be influenced by the developmental stage of individual pups, as well as their general condition. Vocalizations are thought to be modulated by emotional/motivational mechanisms governed by the limbic system (Ehret, 2005). Receptor systems of the brain are the target for modulating neurotransmitters, such as serotonin and dopamine, and other neuromodulators, such as opioids and cholecystokinin, which can both facilitate and inhibit USV production (Barron and Gilbertson, 2005; Ehret, 2005; Hofer, 2010). Interestingly, the profile of neurotransmitters involved in separation vocalization in infant pups is very similar to the neurotransmitter pattern that mediates human anxiety and is also thought to influence separation anxiety in children (Hofer, 2010). USVs of infant mice have become an important phenotypic marker for the characterization of mouse strains for neurodevelopmental disorders, such as autism (Scattoni *et al.*, 2009). The genetic mutant TSC2 affects learning and memory, and also mother–offspring communication and subsequently pup USVs (Young *et al.*, 2010), but a detailed understanding of the link between USV production and neurological development requires further investigation. It remains to be determined whether pups learn from their parents' ultrasonic calls (familial imprinting) or develop dialects, as do many songbirds (Boughman and Moss, 2003).

USV functions in parent–offspring communication

In young house mice USVs have an important function in maternal–offspring communication. Pups emit USVs under potentially threatening conditions, such as isolation from the nest, low temperature, unfamiliar odours, and during rough handling (Branchi *et al.*, 1998), and the USVs of pups initiate

specific maternal behaviours, such as searching and retrieval of pups (Hahn and Lavooy, 2005). In addition to USVs, mouse pups have other calls in the acoustic range, such as a 'wriggling call', which they emit when they are nursed to initiate grooming and other maternal behaviours (Ehret and Bernecker, 1986), and 'pain calls', which they emit as a reaction to being bitten, pinched, or injured, and postpartum sounds when the mother is cleaning them after birth (Ehret, 1975). Interestingly, pups adjust their USV calling rate in different social contexts: calling increases 2–3-fold when an isolated pup has a brief encounter with its dam and gets re-isolated (Shair *et al.*, 2005); however, pups cease emitting USVs when facing unfamiliar – and potentially infanticidal – males (Branchi *et al.*, 1998). In addition to the influence of conspecific interactions on USV production, predation may also play a role in facultative changes in call production. Small mammalian predators (e.g. weasel, mink, cat, and dog) of house mice can detect ultrasonic sounds to some extent (Heffner and Heffner, 1985; Powell and Zielinski, 1989), suggesting that high rates of call production may increase predation risk.

Dams show a stronger retrieval response to their own pups compared to alien individuals (D'Amato *et al.*, 2005), which indicates that USVs play a role in offspring recognition. This finding demonstrates kin recognition in the broad sense (the ability to differentiate kin from non-kin), as well as kin-biased behaviour, but it is unclear if males are able to recognize their own offspring via their USV calls (genetic similarity detection or kin recognition in the narrow sense; Penn and Frommen, 2010). USVs alone are sufficient to stimulate maternal searching behaviour towards the sound source, and female mice prefer USVs and synthesized calls with typical acoustic properties of their own species (reviewed in Ehret, 2005). Interestingly, the sex and developmental stage of offspring influences maternal retrieval behaviours (male pups receive more maternal care than females, and more-developed pups are retrieved sooner than less-developed individuals) (Hahn and Lavooy, 2005), something that may be explained by differences in the calling rate of infant male and female mice (Hahn *et al.*, 1998). Interestingly, maternal responsiveness of a mouse strain is negatively correlated with pup USV calling rate, indicating a close linkage of these behaviours in lab strains (D'Amato *et al.*, 2005; Wöhr *et al.*, 2008). This interaction of maternal response rates and offspring USV production suggests the potential for parent–offspring conflicts, which could be a fruitful area for future research (Box 10.3).

As USV call production is closely associated with thermal regulation, pups' distress USVs may have initially evolved as sounds produced as a by-product of their response to cold, which were evolutionarily co-opted and modified for communication functions. Also, pup USVs may have evolved before USVs in

Box 10.3 Evolution of animal communication

Communication occurs when the action of (or cues given by) one animal influence the behaviour of another (Endler and Basolo, 1998). Both natural and sexual selection can influence both the signal design and the behaviour of the receiver. Sometimes the communication signal evolves based on a pre-existing sensory property of the receiver, which evolved for reasons unrelated to communication. This possibility is referred to as 'sensory exploitation' (Ryan, 1998). As in most signalling systems, information transfer is vulnerable to deception and manipulation (Dawkins and Krebs, 1978) by the sender, though the receiver is expected to attempt to ensure the accuracy of information transfer. Communication is best viewed not as a harmonious exchange of information, but as the focus of an arms race between signallers as manipulators and receivers as 'mind-readers' (Dawkins and Krebs, 1978). This is also true when looking at communication between the sexes. It is no longer assumed that courtship and mating are only cooperative interactions. There is increasing evidence for evolutionary conflicts between the sexes (Holland and Rice, 1998; Arnqvist and Rowe, 2005) as males are typically under stronger selection to maximize mate acquisition and can benefit by feigning higher quality to potential mates. In contrast, females are under stronger selection to mate with superior partners and consequently benefit most from reliable assessment (Trivers, 1972; Clutton-Brock, 2007). When signals are energetically expensive to produce, according to the 'handicap principle' (Zahavi and Zahavi, 1997), they should provide honest information about the signaller's quality.

Sound as a communicating medium is used by several classes of vertebrates and non-vertebrates (Bradbury and Vehrencamp, 1998). Vocalizations are used in intra- and interspecific interactions and have evolved to communicate information about individuals' condition, social state, and quality to potential rivals and mates, as well as to inform listeners about objects (e.g. predators) and events in the environment (e.g. group movement) (Bradbury and Vehrencamp, 1998; Owings and Morton, 1998).

adults, and the sensitivity of females' ears to pups' USVs may have been exploited by males, facilitating the evolution of the courtship USVs in adult males (sensory exploitation) (see 'The evolutionary origins of USVs in house mice' below).

Adult vocalizations

Adult house mice emit USVs to other adult conspecifics (Nyby and Whitney, 1978; Nyby, 1983; Maggio and Whitney, 1985; White *et al.*, 1998; Gourbal *et al.*, 2004). In sexual encounters, male house mice can produce USV calls at high rates, while females emit few or no calls (largely defensive squeaks in the acoustic frequency range) (Wang *et al.*, 2008). USVs can be stimulated by using anesthetized partners or respective urinary cues (Nyby *et al.*, 1977b; Maggio

and Whitney, 1985; Warburton *et al.*, 1989), and the sex of the adult stimulus animal is critical for the elicitation of USVs: male house mice emit USVs when presented with female mice or their urinary scent, and emit few, if any, USVs when presented with male scent (Nyby *et al.*, 1976; Musolf *et al.*, 2010). This sex-specific stimulus response is precisely the opposite for females, but female–female pairs produce USVs at comparable rates (at least during the first minutes of social interaction) (Maggio and Whitney, 1985; Gourbal *et al.*, 2004; Costantini and D'Amato, 2006). Spectral differences of USVs between the sexes have been found in wild *Peromyscus boylii* (Kalcounis-Rueppell, personal communication) and wild-caught singing mice (*Scotinomys* and *Baiomys*, Miller and Engstrom, 2007), though we know of no such comparisons in house mice.

Male USVs: courtship calls

Proximate mechanisms of USV production

Most research on USVs in house mice has been aimed at understanding the influence of hormones and pheromonal regulation in call production. Male ultrasound production is only found in individuals at sexual maturity (*ca.* 55 days) (Whitney *et al.*, 1973; Nyby *et al.*, 1976), when the concentrations of circulating androgens begin to increase, and males begin emitting 70-kHz ultrasounds in response to females, in association with other courtship behaviours (Sales, 1972b; Whitney *et al.*, 1973). Castration reduces adult male calling (Dizinno and Whitney, 1977) and androgen replacement restores it (Nunez *et al.*, 1978; Nunez and Tan, 1984; Warburton *et al.*, 1989), indicating a strong link between the levels of circulating sex hormones and call production. USV calling rate can be used as an indicator of sexual arousal leading to 'reflexive testosterone release', which increases the male's copulatory behaviours (James and Nyby, 2002; James *et al.*, 2006). Recent studies on a receptor knock-out strain suggest that muscarinic receptors involved in neuronal interactions are required for male USV production during male–female interactions, perhaps via their roles in dopamine activation, which imply a potential role in reward, motivation, and cognition (Wang *et al.*, 2008). However, molecular and genetic control of USV production is largely undetermined.

Exposure to female house mice, or female odorants including urine, saliva, and vaginal fluids, provide the most potent sensory cue found so far to elicit males' USVs (Whitney *et al.*, 1974; Nyby *et al.*, 1977b; Guo and Holy, 2007), whereas male urine and control chemicals are relatively ineffective (laboratory mice: Whitney *et al.*, 1974; Nyby *et al.*, 1977b; wild-derived mice: Musolf *et al.*, 2010). Interestingly, males' courtship USVs are a species-specific response: male laboratory mice elicit USVs to fresh female urine of their own strain and a reduced

response to fresh female urine of other strains of *M. domesticus*, but USV production is strangely attenuated when mice are presented with either female rat or human urine (Wang *et al.*, 2008).

Two female pheromones have been identified that elicit male USVs (Sipos *et al.*, 1992). These compounds are only present in female urine after sexual maturity, explaining the lack of male response to the urine of immature females (Whitney and Nyby, 1979; Musolf *et al.*, 2010). One of these pheromones is 'potent' in freshly voided urine, as it elicits USVs from males regardless of males' former social or sexual experience (Sipos *et al.*, 1992); however, this compound is also ephemeral (after 15–18 hours its activity is destroyed by oxidation) (Sipos *et al.*, 1993, 1995). The second pheromone is stable for at least 30 days (Nyby and Zakeski, 1980), but requires male social or sexual experience in order to trigger USVs; naïve males do not respond to this urinary signal. The pheromones in female mouse urine that elicit male USVs may be the same compounds that produce testosterone surges, as they are both non-volatile, ovarian-independent, and regulated directly by the pituitary gland (Nyby *et al.*, 1979; Johnston and Bronson, 1982). Whereas males do not habituate in their USV response when presented with fresh urine from females (eight trials within 23 days; Sipos *et al.*, 1992), aged urine leads to habituation after three or four exposures (Dizinno *et al.*, 1978) and short-term freezing of urine decreases males' USV responses (Hoffmann *et al.*, 2009). These findings provide examples of how males' USVs can be used to examine their ability to detect females' pheromones and sexual attractiveness to different females.

A recent study on laboratory mice (strain B6D2F1, which is an F_1 hybrid between C57B1/6 and DBA2/J) found that male USVs show features of birdsong (Holy and Guo, 2005). The study used a narrow, *sensu stricto* definition for 'song' (Broughton, 1963), and though this label is sometimes debated, this finding opens up many possibilities for future research, which should complement the extensive body of research on bird song. We recently conducted a detailed description of specific call structures and elements, which indicates striking differences between laboratory versus wild-derived house mice (Hoffmann *et al.*, 2012b). Although calls of both groups could be divided into two spectral categories, the calls of wild-derived mice (65 kHz and 103 kHz) appear to be shifted to higher frequencies than those documented in laboratory mice (40 kHz and 67 kHz) (Barthelemy *et al.*, 2004). Our findings support other evidence for genetic influences on USVs, though the evolutionary origin of the differences between domesticated and wild mice remains to be elucidated.

To our knowledge only one study has examined individual variability (or consistency) of males' USVs (Hoffmann *et al.*, 2012a). The USVs of individual, wild-derived male mice were consistent in their spectrographic features, enabling

call classification into respective kin groups and beyond that to the individual level, suggesting that these calls may provide information useful for individual recognition.

It has been suggested that social experience, such as prior physical and sensory interactions with conspecifics, is a prerequisite to elicit males' USV responses to (aged) female urine (Sipos *et al.*, 1992), and that it enhances males' USV responses to female scent (but see Musolf *et al.*, 2010). Exposing males to artificially odorized females results in males subsequently emitting USVs to the artificial fragrance on its own (Nyby *et al.*, 1978). Interestingly, social experience in females has the opposite effect, as increased experience decreases their USV response to other females (D'Amato and Moles, 2001). Although these studies suggest that social learning may play a role in the development and expression of USVs, it is important to note that social deprivation used in these studies (using isolated individuals as controls) could have produced laboratory artefacts, and future studies using animals held under more natural controls are needed. Genetics appear to be another source of individual variation in USVs (Box 10.2). Identifying genes that influence USVs could help to better understand the evolution of USVs, as well as the underlying mechanisms.

Functions of USVs in mate choice

Why do male house mice produce USVs? Early studies concluded that male USVs function to facilitate or coordinate mating, but they did not suggest why females are attracted to these vocalizations. Similarly, male USV have been suggested to reduce females' aggression when the male is approaching the female, though this effect appears to be inconsistent (Bean *et al.*, 1986). Since USVs in house mice are sexually dimorphic with respect to calling rate, androgen-dependent, and produced chiefly by males during mating (triggered by female pheromones), they can be considered to be *secondary sexual characters*, which, like scent-marking (Zala *et al.*, 2004), evolve through sexual selection (Musolf *et al.*, 2010). Males' USVs may evolve through intrasexual (direct male–male competition) or intersexual selection (female choice) or a combination of both. There is as yet no direct evidence that USVs enhance male mating success (through either type of selection), and studies are needed on wild or wild-derived mice in natural or semi-natural conditions to determine the costs as well as the benefits of producing USVs, something which cannot be investigated under laboratory conditions. With the development of improved technologies for measuring USVs, an increasing number of USV studies on wild rodents have been undertaken (*Peromyscus* mice: Kalcounis-Rueppell *et al.*, 2006, 2010; *Scotinomys* and *Baiomys*: Miller and Engstrom, 2007). Previous studies on laboratory mice,

and the few studies conducted so far on wild-derived house mice, provide some insights into the functions of USVs.

As emphasized above, USVs during courtship and mating are produced primarily by males (White *et al.*, 1998), and recent studies suggest these vocalizations play a role in female choice. Females are more attracted to vocalizing than non-vocalizing males (muted by surgical abduction), and male ultrasonic vocalizations prolong the time females spend near potential mating partners (Pomerantz *et al.*, 1983). Studies using recorded USV playback also indicate that females are attracted to males' USVs. In both laboratory (Hammerschmidt *et al.*, 2009) and wild-derived (Musolf *et al.*, 2010) mice, females spent more time near a speaker playing recorded male USVs than a speaker playing only background noise. However, females rapidly habituated to this experimental setting and showed no further preferences when tested in a second trial (Hammerschmidt *et al.*, 2009; Musolf *et al.*, 2010). While females appear to show a preference for males producing USVs, female house mice still copulate with devocalized males when housed in mating pairs (White *et al.*, 1998), indicating that male USVs are not essential for reproduction (at least in cages). Male ultrasonic vocalizations may affect female behaviours during copulation and extend copulation duration, a result documented in hamsters (*Mesocricetus auratus*; Floody and Pfaff, 1977b).

Do USVs provide information about males' quality or compatibility? Males' USVs likely play a role in *sex* and *species recognition* (allowing hybridization avoidance), but these are not likely to be their only functions, as highly complex calls would not seem to be necessary for such roles (simple sex- and species-specific chirps would presumably suffice). Males' courtship USVs might also play a role in *individual* and *kin recognition* (Musolf *et al.*, 2010), and females may use these vocalizations to assess male compatibility, to obtain direct or indirect genetic benefits for their offspring. We have recently shown that wild-derived females prefer USV playback from non-kin males to those produced by their male siblings, providing evidence that USVs alone are sufficient for kin discrimination (Musolf *et al.*, 2010).

USVs may also enable females to assess males' quality, such as their social status (see below) and condition (e.g. health and resistance to infectious diseases), as do chemosensory signals (reviewed in Penn and Potts, 1998). There is much evidence to support the handicap hypothesis of costly signalling, even if signals do not necessarily need to be costly to be honest (Maynard Smith and Harper, 2003). Ultrasonic calling is assumed to be energetically costly (Nyby and Whitney, 1978), and there are several reasons to suspect that males' USVs may be costly to produce in terms of time as well as energy, though we know of no direct evidence in house mice. Food-deprived mice produce USVs at lower rates than those produced by well-nourished individuals (Moles and D'Amato,

2000). Similarly, malnutrition leads to a reduction of specific call types in rat pups (Barron and Gilbertson, 2005). The production of USVs may be particularly costly for food-restricted animals, a hypothesis suggested in several empirical studies. The production of USV may also reduce the time available for foraging and thus lead to courtship/foraging tradeoffs, a pattern found in birds (reviewed in Gil and Gahr, 2002). The investigation of such tradeoffs requires studies on wild or semi-natural populations of mice. If USV production can be shown to impose time and energy tradeoffs, USVs might provide honest signals indicating the caller's condition, signals that could be used in mate-choice decisions.

While there is generally a negative relationship between body size and the frequency of sound production (low frequencies require large body structures) (Pye, 1979), this relationship does not appear to hold for USV production in rodents. Although some studies support a relationship between body size and call frequency (e.g. USVs emitted during male–male encounters: calls of field voles (*Microtus agrestis*) have higher fundamental frequencies than those of smaller common voles (*Microtus arvalis*) and bank voles (*Myodes glareolus*); Kapusta *et al.*, 2007), others do not (e.g. exploratory ultrasonic calls of both male and female wood mice (*Apodemus sylvaticus*) are higher in frequency than those of the larger yellow-necked mouse (*A. flavicollis*); Hoffmeyer and Sales, 1977). No studies to our knowledge have yet investigated intraspecific variation in body size and its relationship to USV production.

Males' courtship USVs do not necessarily function to provide *information* to females, as much as to persuade and manipulate them (as with all advertisements), and future research should examine their potential role from a sexual conflict perspective (Box 10.3).

Neither laboratory (Nyby *et al.*, 1976) nor wild-derived males (Musolf *et al.*, 2010) appear to use USVs in intrasexual communication, suggesting that male USVs are not currently under intrasexual selection. This pattern stands in striking contrast to the pattern observed in birds, where song plays a key role in territorial defence and direct male–male competition, and songs are displayed regularly if not exclusively to other males (Collins, 2004). However, this sexual bias does not necessarily mean that USVs in mice do not play a role in male–male competition. Nyby *et al.* (1976) found that subordinates utter fewer USVs than dominants in the presence of a female, whereas D'Amato (1991) found the opposite: subordinates called more in the temporary absence of the dominant male, though subordinates' calling rate was inhibited by exposure to urine of the dominant males interacting with a female. Social defeat has also been shown to reduce males' USVs (Lumley *et al.*, 1999). Thus, USVs appear to provide information on a male's social status, as found with other mammal vocalizations (Fischer *et al.*, 2004; Behr *et al.*, 2006; Vannoni and McElligott, 2008), and even humans (Sell *et al.*, 2010), and thus affect

the behaviour and physiology of other males (e.g. differences between dominant and subordinate male mice: scent marking behaviour (Hurst, 1990), urine excretion (Drickamer, 1995), urine chemistry (Novotny *et al.*, 1990), and aggression (Hurst *et al.*, 1993)). Future studies are needed to determine whether and how male USVs might play a role in male–male interactions.

Female USVs

Proximate mechanisms

There have been relatively few studies of female house mouse USVs and they remain relatively poorly studied. The few studies so far suggest that female USVs are also hormonally modulated, as their production varies during the oestrous cycle, and sexually receptive females emit fewer USVs than non-receptive individuals in the presence of a female intruder (Moles *et al.*, 2007). In contrast to males, the control of USVs in females does not appear to be androgen-dependent. Ovariectomized females began to call to stimulus females at similar rates as long-term gonadectomized males when receiving testosterone treatment (Nyby *et al.*, 1977a). However, USVs were accompanied by mounting bouts in this study, suggesting that females demonstrated inappropriate male-typical behaviour, which does not take place in natural female–female encounters. Further studies are needed to understand the development and proximate mechanisms controlling sex differences in USVs in house mice.

Functions of female USVs

As female USVs have received so little attention, their adaptive function remains a mystery. In general, sexual ornaments in females have often been considered to be a by-product of selective pressures on male ornamentation (an analogue of males' nipples); but evidence suggests that female ornaments have often evolved independently and may have important functions in competitive situations (Amundsen, 2000). Since female ultrasonic vocalization appears to occur principally during female–female social investigations (Moles *et al.*, 2007), this behaviour may play a role in the establishment of social dominance among females. The role of female USVs has not yet been investigated in courtship or male mate choice. It has been suggested that female USVs are important for sex recognition (Wysocki *et al.*, 1982); these calls may also play a role in species, group, kin, and individual recognition (Musolf *et al.*, 2010). Indeed, it has been shown that female USVs can be used to indicate social recognition: resident females recognize familiar individuals intruding into their territory, and the rate of USVs rapidly decreases in such situations, a pattern that differs from that observed with novel females, whereas their olfactory behaviour did not differ in these situations (D'Amato and Moles, 2001). Social recognition ability is known to

depend on the time interval between encounters (the ability disappears after 60 min; D'Amato and Moles, 2001), and recognition also depends on the age of the female (older females having shorter memory spans; Moles *et al.*, 2007).

Besides individual recognition, female USVs may function to communicate additional condition-dependent information. Moles and D'Amato (2000) found that females emitted a higher number of USVs during the first minute of social interaction when the intruder had previously fed on a palatable food in contrast to one fed on unpalatable food. Whereas the results of these few studies are interesting, a more systematic approach to the study of female USVs is needed in order to understand their potential function in social communication.

The evolutionary origins of USVs in house mice

There have been no comparative studies on the evolutionary origins of USVs in mammals to our knowledge, though USVs have been detected in at least 50 species in 30 genera, all from two suborders, the Sciuromorpha (two families) and Myomorpha (eight families) (see overview in Sales, 2010). There are no single species-specific frequency bands or patterns that would permit species recognition (Bradbury and Vehrencamp, 1998), but there are some differences in the form and frequency of USVs produced in different groups. Infant house mice and other members of the Muridae appear to call at higher frequencies than pups of the genera *Rattus* and *Thamnomys* (Sales, 2010). The pattern of frequency modulation, which also appears to vary among taxa, seems to be more common in murid rodents than in cricetids. Harmonic elements (i.e. additional signal(s) whose frequency(ies) are integral multiples of the reference signal) have not been detected in all species, but these differences may, to a certain extent, reflect the methods of recording and reporting of the calls (Sales, 2010).

Studies on USVs in Norway rats (*Rattus norvegicus*) show interesting similarities and differences compared to house mice. In rats, adult male USV calls are emitted during reproductive and agonistic situations, and adolescent rats call during play activities and even in response to human 'tickling' (Brudzynski and Ociepa, 1992), in addition to which they also have specific alarm calls (Brudzynski, 2007). Both male and female rats emit ultrasonic calls during sexual encounters (White and Barfield, 1990), as with golden hamsters (Floody and Pfaff, 1977a), collared lemmings (Brooks and Banks, 1973), and different vole species: *Microtus arvalis*, *M. agrestis*, *M. oeconomus*, *Myodes glareolus* (Kapusta *et al.*, 1999, 2007; Kapusta and Sales, 2009). In rats, male pre-ejaculatory vocalizations solicit female darting, possibly coordinating male and female reproductive behaviour (Thomas *et al.*, 1982), and facilitate lordosis in females (White and Barfield, 1990). Male and female voles elicit USVs both in intra- and intersexual

encounters (Kapusta *et al.*, 1999, 2007; Kapusta and Sales, 2009). Therefore, compared with rats and other rodents, house mice USVs appear to be produced under a more restrictive set of social conditions.

Two hypotheses have been developed to explain the evolutionary origins of USVs in rodents. The first of these, the *sensory exploitation hypothesis*, suggests that USV communication originally evolved in pups, where it plays a role in mother–offspring interactions, and was later co-opted and modified for communication in adults (Ehret, 2005). Infants of virtually every species of mammal, as well as birds, vocalize when separated from their home nest, conspecifics, and most clearly their mothers. Newborn mouse pups need their mothers not only for protection and food provisioning, but also for thermoregulation. It has been suggested that laryngeal braking associated with thermoregulations may be responsible for USV production in pups, suggesting that USVs may have arisen originally as an acoustic by-product of physiological adaptations to recover from hypothermia in juvenile rodents and that the role of these vocalizations in maternal–offspring communication may have followed their initial role in thermoregulation.

Both male and female rodents produce ultrasounds in various situations (e.g. Sales, 1972a; Nyby, 1983; Maggio and Whitney, 1985; Brudzynski *et al.*, 1993), and though the acoustic properties of adult USVs may differ from those of pups in several respects (Liu *et al.*, 2003), they seem to reflect states of arousal and motivation/emotions similar to those of infant rodents and may have evolved from infant calls (Ehret, 2005). It is interesting to consider the possibility that once USVs became established in maternal offspring communication, this sensory adaptation may have opened an opportunity for these cues to be co-opted during courtship, with males producing USVs at high rates being favoured by females (sensory drive or sensory exploitation). Although the opposite evolutionary sequence is also possible, with pups exploiting the pre-existing USV detection ability of their mothers, this scenario seems less probable since pups are deaf for their first days of life (Ehret, 1983).

A second – though not mutually exclusive – hypothesis suggests that the evolution of USVs may have been mediated by *predator avoidance*. Many predators (and parasitoids) eavesdrop on the communication signals of their prey, and USVs might have initially evolved, or been reinforced, as a way to evade predator detection (Hofer, 2010). The ability to detect USVs requires complex morphological changes in the inner ear bones and additional sensory hair cells. While ultrasonic calls are not entirely cryptic to predators, many predators cannot detect the full range of USV and USV alarm calls that are utilized by some rodents (i.e. rats; Brudzynski, 2007). Further, particular physical characteristics of ultrasounds (greater directionality, greater attenuation, greater scattering, decreased

localizability and suitability for communication in underground burrows) make ultrasound a superior alternative to sonic vocalization for short-range communication, particularly in critical situations.

Conclusions: potential future directions

USVs have been studied in laboratory rodents since the late 1950s, and the recent discovery that USVs of male house mice have features of song (Holy and Guo, 2005) is sure to stimulate even more research into this communication mode that is cryptic to humans' senses. To understand the proximate mechanisms controlling USV communication and their ontogeny, more neuro-ethological studies are needed. For example, to determine how female pheromones activate male courtship USVs, we will need to understand how olfactory signals can be processed via receptors and transferred to the brain for USV production.

Vocal communication is not the only information channel animals rely on, of course, and it would be particularly interesting to know how mice integrate information from multiple sensory modalities, such as olfactory and acoustic signals. Such *multimodal sensory integration* is a challenging area of research and more accurately reflects natural situations in which animals receive information on different sensory channels. Neuro-ethological and other studies on mechanisms of USV production could benefit by considering more ecological contexts, as some behavioural paradigms developed in the laboratory are non-existent in the wild (Wolff, 2003).

While the structure and function of adult male USV has been studied in detail, a lack of comparable studies on adult female house mice means we still have a relatively poor understanding of female USVs. Future studies (e.g. comparing intersexual differences in call characteristics) are needed to improve our knowledge of female ultrasonic communication.

To understand the functions of USVs, studies on wild house mice are crucial, especially in an ecological context (behavioural ecology; ecological genomics). Our research on the USVs of wild-derived house mice provide a new perspective, as they indicate that males produce USVs as part of their courtship display to attract females (Musolf *et al.*, 2010). USVs are individually consistent and distinguishable (Hoffmann *et al.*, 2011a) and females could use them for kin recognition (Musolf *et al.*, 2010). Moreover, as previously mentioned, domesticated strains of mice have low individual variability and show many differences from their wild counterparts in behaviour (Crawley *et al.*, 1997) and life history traits (Miller *et al.*, 2002) due to inbreeding and hybridization of different *M. musculus* subspecies (Wade and Daly, 2005). Further, rearing house mice in

standard laboratory conditions reduces the diversity of their USVs compared to rearing in socially and environmentally enriched conditions (mixed-genders, nest boxes, shelters) (Portfors, 2007), and therefore more such studies are needed to investigate the effect of rearing conditions and laboratory artefacts. Nevertheless, such findings strongly underscore the importance of studying USVs in outbred, wild mice, and also under wild, or at least semi-natural, conditions.

Wild mice likely exhibit greater diversity and complexity in their USVs than laboratory mice, as has been demonstrated in *Peromyscus californicus* (Kalcounis-Rueppell *et al.*, 2010), and studies on wild populations combining USV characteristics with genetic analyses might help us to understand the evolution of these courtship displays. More detailed analyses on male courtship USVs should help to assign call characteristics to specific functions, which will be needed in order to determine what kinds of information are conveyed in male USVs. Moreover, future studies are needed to examine whether sexual conflict plays a role in USV communication (e.g. males' USVs may alter females' sexual maturity, reproductive cycles, and pregnancy maintenance, as with chemical signals). Manipulation of call parameters and playback experiments make it possible to experimentally test such functional hypotheses. Future studies are needed to investigate *receivers*, as well as senders, both for sensory (hearing) mechanisms, and the selective pressures to detect USVs. Such studies have already been conducted in some detail for mother–offspring communication in laboratory mice both on females' perceptional variation (Ehret and Schmid, 2009) and mother–pup interactions (Wöhr *et al.*, 2010), though not for male courtship calls and detection by females.

Finally, as we have previously emphasized, molecular genetic tools provide great opportunities for determining the genetic basis of USVs and the evolutionary origins of these calls (Box 10.2). Such genetic dissection would complement studies on other mammals and even songbirds. As more studies begin to use USV behaviour to characterize phenotypic effects in knock-out strains (Scattoni *et al.*, 2009), such studies will provide new insights into the genetic mechanisms and pathways controlling USV behaviour. Although there is already some evidence that USVs are heritable in laboratory strains, virtually nothing is known about the genetics or evolution of USVs in wild house mice. Integrating studies on well-characterized strains of laboratory mice with detailed investigations of the functions of these vocalizations under ecologically relevant situations should continue to provide new insights into this exceptional form of auditory communication.

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